THE EFFECTS OF ACUTE PROGRESSIVE HYPOXIA ON THE RESPIRATION RATE OF THE CHINESE CRAB

Eriocheir sinensis

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Abstract The effects of acute progressive hypoxia on the respiration rate of the Chinese freshwater crab, *Eriocheir sinensis*, acclimated at three temperatures were investigated with a closed respirometer. *E. sinensis* can maintain its respiration rate down to the critical point (Pc) and from this point its respiration rate declines rapidly, reaching zero at a lower ambient oxygen concentration called the zero respiration oxygen concentration. Because of this, a new hyperbolic equation is introduced to express the relationship between respiration rate and ambient oxygen concentration. A new method for calculating the Pc value is also developed. The Pc values for *E. sinensis* at 20—35℃ range from 1.92—3.47 mg / l.

Key words: *Eriocheir sinensis*, Progressive hypoxia, Respiration rate

Introduction

In terms of an animal's respiration response to hypoxia, most aquatic animals have traditionally been classified as oxyregulators or as oxyconformers (Prosser, 1962). It is well known that species frequently encountering hypoxia in their natural habitats, such as the intertidal species and the burrowing species, tend to be oxyregulators (Teal *et al.*, 1967; Thompson *et al.*, 1969; Marker *et al.*, 1987).

A distinctive feature for oxyregulators is the critical point, Pc, which refers to the oxygen concentration at which an oxyregulator becomes sufficiently stressed by

hypoxic condition that its respiration rate becomes dependent upon ambient oxygen concentration. It is valuable to develope a simple and reasonable method for calculating Pc.

The Chinese freshwater crab, *Eriocheir sinensis*, a burrowing species, is widely distributed in the Yangtze basin of China. It is likely that this animal encounters periods of environmental hypoxia in the burrow, particularly in summer (Chen, 1932). However, found that *E. sinensis* was an almost absolute oxyconformer, which seems unlikely for this species. The aims of this investigation are to (1) carefully reexamine the response of the respiration rate of this animal to hypoxia; (2) develope a new method for Pc calculation if *E. sinensis* is an oxyregulator.

Materials and Methods

All the experimental animals ranging from 21—92 g were purchased at the Shanghai free market. They were maintained in aerated aquaria and acclimated to 20, 25 or 35℃ for at least three weeks prior to formal testing. They were fed two to three times a week with earthworms, freshwater clams and small fish. Food was withheld during the experiment.
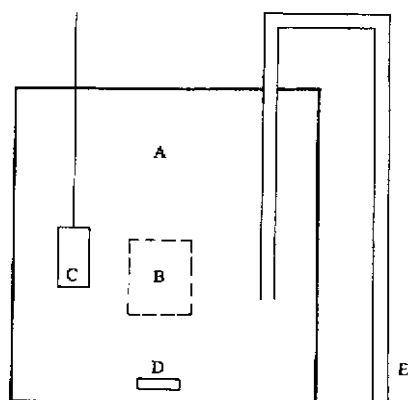

Fig. 1 The structrue of the closed respirometer used in this investigation

A. closed container;

B. holed animal cage;

C. thermoregulator;

D. magnetic stirrer;

E. sampling tube

The structure of the respirometer is shown in Fig.1. The animal was placed in a small ventilated cage which was submerged in a closed plastic container. In the cage, there was a small amount of room for the animal to move. The water in the container was thermoregulated and a magnetic stirrer was used to prevent stratification of the oxygen. The volume of water in the container varied from 9.3—25 liters, depending on the size of animal (approximately 300 times the wet weight of the animal). It was found that with this proportion the oxygen concentration in the container could be de-

pleted to one-fifth saturation level within 7—15 hr. The experimental animal was allowed to acclimate to the respirometer for 2 hr, during which the container was bubbled with air. After this, the air pump was turned off, the container sealed and the decline in oxygen concentration was measured by analysing the oxygen content in 60 ml water (Winkler's method) sampled at different times. The respiration rate was caculated by :

$$R = \triangle P * V / M\triangle t \ (\mu gO_2 / gmin)$$

Where $\triangle P$ = difference in oxygen concentration; V = volume of water in the container; M = animal's wet mass; $\triangle t$ = length of time. The respiration rate thus calculated was considered to be the respiration rate at the mid—point oxygen concentration of the interval.

The hyperbolic equation $R = (-a+bP) / P$ (a, $b > 0$) was used to describe the relationship between respiration rate and ambient oxygen concentration, where R = respiration rate ($\mu gO_2 /$ gmin); P = oxygen concentration (mg / L); a and b are constants calculated from the linear form of the above equation, i. e. $R = -a(1 / p)+b$. Ratio a / b, an index for oxyregulative capacity, reflects the dependence and independence of the animal's respiration responses to hypoxia——— the higher the ratio, the more the respiration rate depending on ambient oxygen concentration. The critical point (Pc) was calculated by $Pc = \sqrt{aPs / b}$, Where Ps = the saturated oxygen concentration at a specific temperature. The reasons for developing the hyperbolic equation above and the calculation of Pc are given in the "Discussion" section.
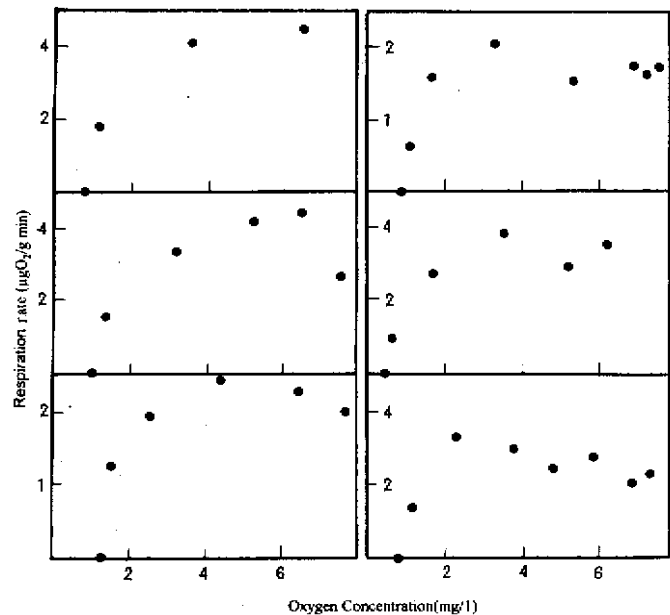

Fig. 2   Respiration rate of *Eriocheir sinensis* as a function of ambient oxygen concentration at 20℃

Results

A total of 22 experiments relating the respiration rate of *E. sinensis* to oxygen concentration were carried out at three experimental temperatures, 20, 25 and 35℃. The regression equations of individual experiments are given in Table 1. The significant levels for regression equations are mostly satisfactory, suggesting a good fit between experimental data and the hyperbolic equation adopted in the present study.

Table. 1   Summary of regression equations of the respiration rate of *E. sinensis* as a function of the ambient oxygen concentration at different temperatures, and the subsequent Pc and a / b values

Temperature (℃)	Wet Mass (g)	$R = -a(1/P)+b$ (μgO_2 / gmin)	N	R	SL	a / b	Pc (mg / L)
20	45.2	$R = (-1.656+2.027P)/P$	8	0.837	* *	0.817	2.74
	50.8	$R = (-4.604+5.223P)/P$	4	0.998	* * *	0.882	2.84
	43.7	$R = (-4.842+4.647P)/P$	6	0.908	*	1.043	3.09
	66.2	$R = (-3.284+2.893P)/P$	6	0.926	* *	1.153	3.23
	35.5	$R = (-1.970+3.968P)/P$	6	0.979	* * *	0.496	2.13
	25.0	$R = (-2.540+3.141P)/P$	8	0.747	*	0.808	2.72
25	49.6	$R = (-7.793+5.415P)/P$	8	0.960	* * *	1.439	3.47
	52.8	$R = (-2.015+2.758P)/P$	11	0.603	*	0.731	2.47
	90.9	$R = (-3.861+4.285P)/P$	6	0.687		0.901	2.75
	89.6	$R = (-3.247+4.738P)/P$	6	0.851	*	0.685	2.40
	69.0	$R = (-3.408+4.090P)/P$	7	0.925	* *	0.833	2.64
	50.1	$R = (-1.523+3.237P)/P$	7	0.956	* * *	0.471	1.99
	35.2	$R = (-2.428+3.527P)/P$	5	0.988	* *	0.689	2.40
	30.0	$R = (-2.753+3.872P)/P$	5	0.806		0.711	2.44
	21.0	$R = (-2.366+4.186P)/P$	5	0.903	*	0.556	2.18
	28.0	$R = (-1.560+3.560P)/P$	6	0.841	*	0.438	1.92
35	79.8	$R = (-5.365+5.822P)/P$	5	0.870		0.922	2.56
	88.7	$R = (-3.137+4.330P)/P$	7	0.845	*	0.725	2.27
	92.0	$R = (-3.189+4.661P)/P$	7	0.958	* * *	0.684	2.20
	37.0	$R = (-3.723+4.565P)/P$	7	0.987	* * *	0.815	2.41
	31.0	$R = (-1.797+3.062P)/P$	4	0.877		0.587	2.04
	29.5	$R = (-7.002+5.462P)/P$	7	0.854	*	1.282	3.02

N, number of respiration data; R, correlation coefficient; SL, significant level.

* P<0.05;   * * P<0.01;   * * * P<0.001

The responses of respiration rate of six animals to declining oxygen concentration at 20℃ are displayed in Fig. 2. All these animals demonstrate a relatively high degree of oxyregulation, and can maintain their respiration rates at a constant level down to an ambient oxygen concentration of 2.13—3.23 mg / L, below which their respiration

rates go down drastically, reaching zero at an oxygen concentration of about 1 mg / L. The oxygen concentration at which an animal's respiration rate is equal to zero is termed the zero respiration oxygen concentration. Since the a / b values of these six animals ranging from 0.496—1.135 well represent the a / b values of all the experimental animals varying from 0.438—1.439 (Table 1), it is safe to conclude that *E. sinensis* is a good oxyregulator. The Pc values for *E. sinensis* at 20—35°C range from 1.92—3.47 mg / L (Table 1).

Discussion

E. sinensis was found to be a good oxyregulator, which is not consistent with Chen (1932)'s results. However, this discrepancy is not unusual as respiratory behavior under hypoxia can be changed by an animal's activity. Taylor (1976) found that inactive *Carcinus maenas* was a good oxyregulator, but under handling stress it became an oxyconformer. Negative results obtained by Chen can be attributed to the following factors. Firstly, Chen did not clarify whether or not animals were allowed to acclimate to experimental conditions after being weighed and measured so it was possibel that the animals were still in an active state during formal recordings of respiration rate. Secondly, the amount of water in the respirometer was only one hundred times the animal's weight, just one third of the water volume used in the present study, and the small volume of water might cause rapid decline in oxygen concentration, making the oxyregulation portion of the R—P curve invisible.
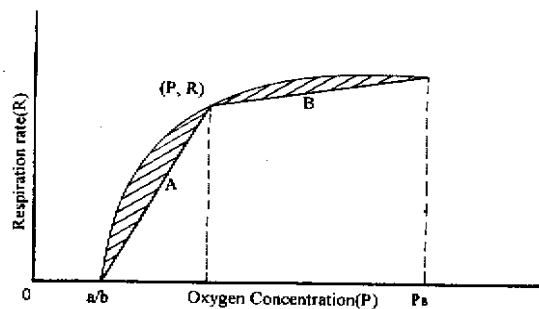

Fig. 3 Curve for the hyperbolic equation $R = (-a+bP) / P$ and two approximating lines, A and B, with A representing dependent phase and B representing independent phase. Ps = oxygen saturation concentration

Tang (1933) used a simple hyperbolic equation $R = P / (a+bP)$ to describe an animal's respiration rate as a function of ambient oxygen concentration. However, this hyperbolic equation conflicts with the fact that zero respiration rate does not necessarily occur at zero oxygen concentration. For example, zero respiration rate in *E. sinensis* is not reached at zero oxygen concentration but rather at about 1 mg / L

at 20℃ . So the authors here introduce another hyperbolic equation, R= (−a+bP) / P, (a, b>0), which does not pass through the original point and has a typical curve as displayed in Fig. 3. Table 2 shows comparisons of regression results from these two hyperbolic equations. Obviously, the equation introduced by Tang (1933) is not applicable in the present investigation since it leads to a negative value for b in some cases (Table 2). Hence, the hyperbolic equation introduced here may be a better expression of an animal's respiration rate as a function of ambient oxygen concentration.

Tab. 2 Comparisons of a and b values from two different regression equations. Both equations based on the same respiration data of *E. sinensis* at 25℃

Regression Equation		1	2	3	4	5	6	7	8	9	10
R = (−a+bP) / P	a	7.793	2.015	3.861	3.247	3.408	1.523	2.428	2.755	2.366	1.560
	b	5.415	2.758	4.285	4.738	4.090	3.237	3.527	3.872	4.186	3.560
R = P / (a+bp)	a	4.085	0.899	8.624	0.818	1.469	1.918	1.340	1.068	0.596	0.348
	b	−1.230	0.238	−2.787	−0.022	−0.154	−0.416	−0.118	0.004	0.045	0.208

Since the Pc point is not necessarily a sharp point, some authors simply identified this by giving a Pc zone on R−P curve (Taylor, 1976; Shumay, 1981; Teal et al., 1967). The shortcoming of this method is that it makes quantitive comparisons among species and within species difficult. It is essential to develope a method to obtain a concrete Pc point which can well represent the Pc zone on the R−P curve. Recently, Kapper et al. (1987) and Villareal (1990) have introduced two iterative methods. Both are statistically reasonable, but they are a bit complicated, and in some cases, investigators have experienced difficulties. For example, Kapper et al. (1987) did not observe any correlation between an animal's oxyregulative capacity and the Pc value, which disagrees with the fact that a good oxyregulator should have a low Pc value (Herried, 1980). In order to simplify the procedures for the Pc caculation and overcome possible discrepancies as met by Kapper et al. (1987), the authors here develope a new method to calculate the Pc value, which is based on the assumption that the hyperbolic curve of equation R = (−a+bP) / P can be approximated by two lines, A and B (Fig. 3), with A representing oxyconformation and B representing oxyregulation. The best approximation happens when the sum of the shaded area (S) in Fig. 3 is minimal. We have:

$$S = \int_{a/b}^{P} (R - RA)dP + \int_{P}^{Ps} (R - RB)dP \tag{1}$$

given s=0, and solving the subsequent equation, we have:

$$P = Pc = \sqrt{aPs / b} \tag{2}$$

Equation 2 means that the Pc valus is in proportion to ratio a / b, i. e., the low-

er the ratio a / b or the better the oxyregulation, the lower the Pc value.

The critical oxygen concentrations obtained for *E. sinensis*, ranging from 1.92—3.47 mg / L at 20—35℃, suggest that, in the aquaculture of this species, the oxygen concentration in the water cannot be lower than the above values for the well—being of the animals.

Acknowledgements

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急性逐步低氧对中华绒螯蟹呼吸率的影响

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摘要　本文用密闭式呼吸仪研究了急性逐步低氧对中华绒螯蟹(又名河蟹)呼吸率的影响。研究发现，河蟹是一种很好的氧调动物。当溶氧在一定范围内下降时，河蟹能维持其呼吸率不变，直到临界氧浓度，或临界点。而后，随着溶氧的进一步下降，其呼吸率迅速降低，并在溶氧未至零值时，呼吸率为零。作者将呼吸率为零时的氧浓度称为零呼吸氧浓度，或零呼吸点。鉴于零呼吸点的存在，作者提出了一种能更好地描述水生动物呼吸率和溶氧关系的双曲线方程，并在此基础上，推导出一种更简便、合理的临界氧浓度计算方法。在 20—35℃，河蟹的临界氧浓度范围为 1.92—3.47 mg／L。

关键词：中华绒螯蟹，急性逐步低氧，呼吸率